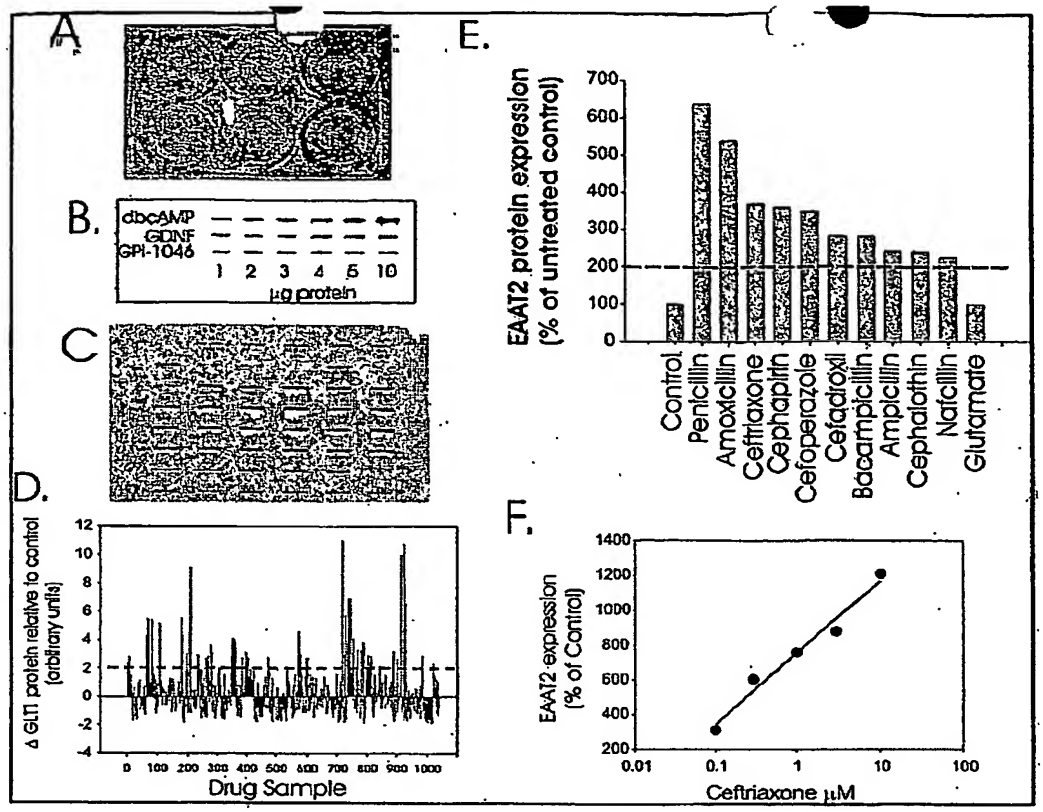

Figure 2. Screen of 1040 FDA approved drugs reveals β-lactam antibiotics as inducers of EAAT2 protein. (A) Spinal cord cultures are incubated with compounds for 3 days. (B) Sample slot blot from tissue homogenates validating the increased in EAAT2 expression seen with increasing controls known to occur with dibutyryl cyclic AMP,GDNF or the neuroimmunophilin GPI-1046. All three compounds (dbcAMP, GDNF and GPI-1046,) induced a large increase in EAAT2 expression, after three days in culture. (C) Typical data slot blot of EAAT2 protein from an early screen of various agents. Every blot included control- untreated tissue and a positive control from panel B. (D) Screening results from the NIH-NINDS Custom Collection screen of 1040 FDA approved compounds. Height of the bars reflects increased EAAT2 protein expression relative to untreated controls. Each blot included at least one untreated control and one known positive control. (E) β-Lactam antibiotics were highly represented in the most potent compounds. These compounds were able to increase EAAT2 protein expression up to 7 fold compared to untreated control cultures, after a 7 days chronic treatment. (F). Dose response analysis for ceftriaxone, revealing EC_{50} 3.5uM for EAAT2 expression.

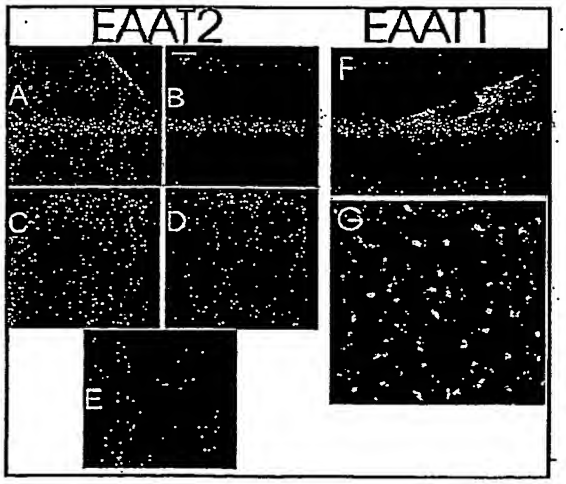

Figure 3. Generation of Promoter reporter transgenic mice. A-E. EAAT2 promoter (E2P)-eGFP mouse brain at 2 weeks of age. Wide spread expression of the reporter in astrocytes throughout the brain parenchyma. F. Astrocytes from EAAT1-Bac promoter-eGFP reporter and (G.) cortical expression of EAAT1 Bac-eGFP reporter mice.

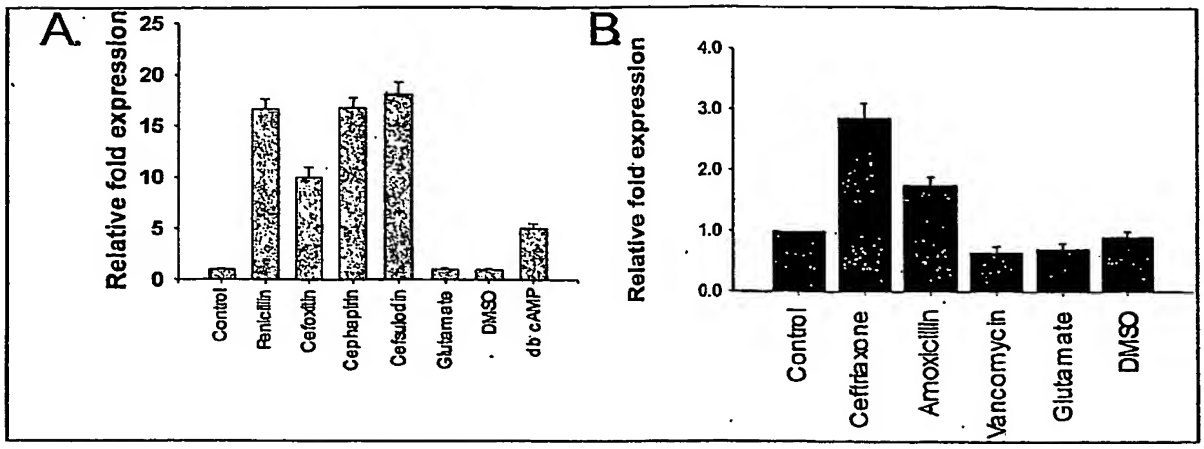

Figure 4. Promoter Reporter Analysis. β-Lactams activate EAAT2 promoter. In both Cos7cells (A) and in (B) human astrocytes transfected with the EAAT2 promoter-eGFP reporter, β-lactam antibiotics (10uM) markedly activate the EAAT2 promoter, while controls such as glutamate has no effect. The known activator, dibutyryl cyclic AMP, has a consistent, but smaller effect. The activation was also dose dependent (not shown).

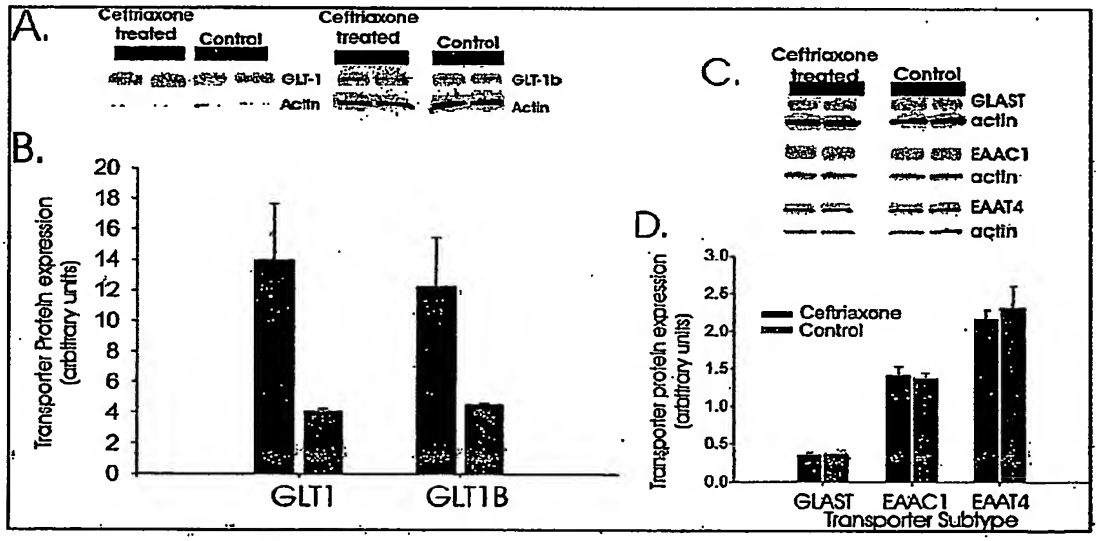

Figure 5. Ceftriaxone induces expression ofGLT1 and GLT1b—but not other proteins, in vivo. Rats were injected (ip) daily for 5 days with ceftriaxone (200mg/kg)- a dose known to produce low micromolar brain concentrations. Hippocampal levels of GLT1 protein (A, by western blot) and its active splice variant, GLT1b were consistently elevated (n=5) by at least 3 fold (B). The expression of the astroglial glutamate transporter GLAST, and the neuronal transporters EAAC1 and EAAT4 were unaffected by this treatment (C and D). The constitutive protein, actin was also unaffected (Panel A and C).

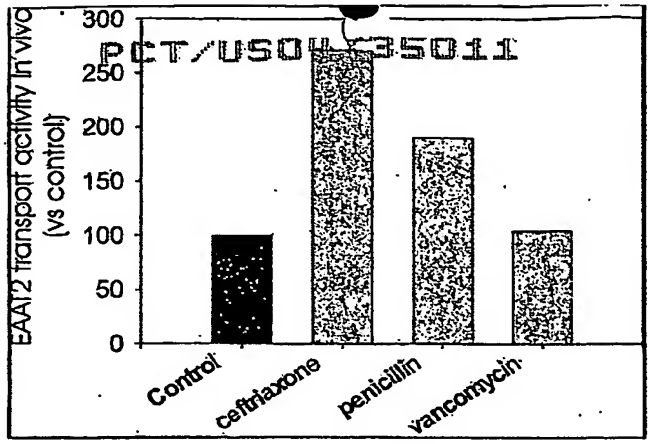

Figure 6. β-lactam antibiotic ceftriaxone and penicillin administration leads to a functional increase in glutamate transport. Daily treatment with ceftriaxone or penicillin (200 mg/kg, 5 days) not only increased GLT1 protein (figure 3), but also increased GLT1-mediated glutamate transport, as determine by ^{3}H-glutamate transport assays (in the presence/absence of dihydrokainate- to measure GLT1- specific transport). The non-β-lactam antibiotic vancomycin, did not increase glutamate transport activity.

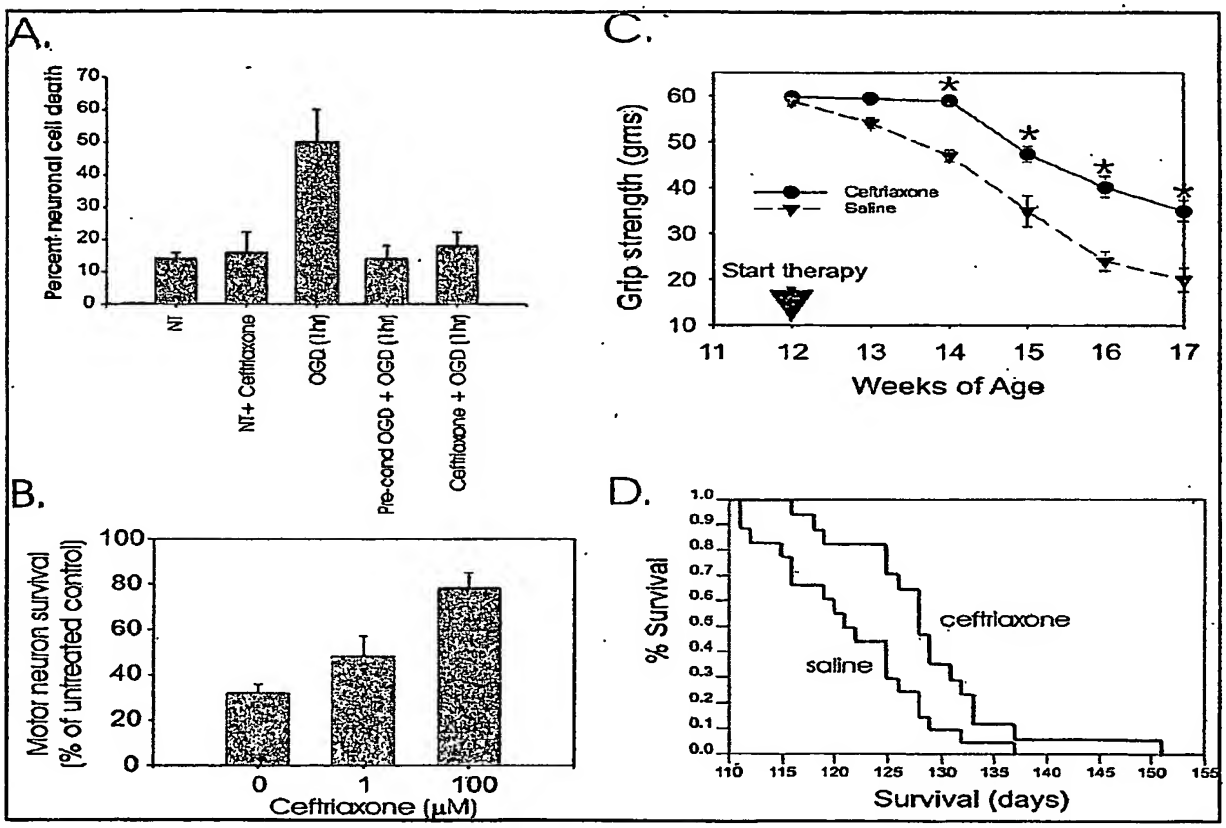

Figure 7. Neuroprotection by Ceftriaxone.
In Vitro Models (A). Ischemic Tolerance. Oxygen glucose deprivation (OGD) of cortical neurons leads to reliable cell death; while preconditioning with brief OGD is protective. Similar protection is afforded by ceftriaxone (1μM) pretreatment. **(B) Motor neuron degeneration.** Ceftriaxone prevents motor neuron degeneration in vitro. Chronic treatment of spinal cord organotypic cultures with the glutamate transport inhibitor threo-hydroxyaspartate leads to loss of >50% motor neurons (point 0, above). Co-treatment with ceftriaxone prevents this excitotoxic loss of motor neurons.
In Vivo Model- G93A SOD1 ALS mice. (C). Ceftriaxone therapy (200 mg/kg daily i.p.) delays loss of muscle strength in G93A SOD1 mice. Therapy was initiated at disease onset (approx 12 wks age). Asterisks indicate significant difference from saline controls ($P<0.05$) at each time point. (D). Ceftriaxone treatment increases survival in G93A mice, when treatment was initiated at disease onset (12 weeks age). For panel C+D, n=20 saline, n=20 ceftriaxone group.